Remarks

Claims 4, 5, 8, 30, 31, 34 and 55 have been cancelled, and claims 1, 6, 7, 20, 22-25, 27-29, 32, 33, 49, 51, 52, and 54 have been amended, both without prejudice to Applicants presenting the cancelled subject matter in any future application. These amendments are presented to comply with one of the election of species requirements (the soluble TNF receptor species). In addition, dependent claims 56-61 have been added. The new claims are fully supported by the application at, for example, page 13, lines 1-4 (for claims 56-58) and page 6, line 36 (for claims 59-61). No new matter or other issues are presented by these dependent claims as they merely recite specific embodiments of already claimed subject matter. Applicants respectfully request their entry into the instant application.

The sole rejection of the pending claims is under 35 U.S.C. § 103. In particular, Claims 1-7, 9-11, 14-27, 30-33, 35-37 and 40-55 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Merli et al., Analytical Biochemistry, Sept. 1, 1995, Vol. 320, No. 1, pages 85-91, and further in view of Beutler et al., U.S. Patent No. 5,447,851, Sept. 5, 1995, Grossenbacher et al., U.S. Patent No. 5,661,001, August 26, 1997, Purchio et al., EP 0 293 785, Dec. 7, 1988, and Thomas, U.S. Patent No. 5,879,673, March 1999. The Examiner states that Merli teaches optimization of refolding of soluble tumor necrosis factor type I produced in E. coli, and also the production of soluble TNF receptor in CHO cells (glycosylated), but that Merli does not teach an Fc fusion or cysteine as the reduction/oxidation coupling reagent. Beutler is cited for teaching the extracellular domain of the 55 kD TNF receptor fused to Fc, production in mammalian cells, protein A purification and chromatography, and use of the recombinant TNF receptor to treat various disorders. Thomas teaches that oxidized and reduced glutathione or cysteine can be the redox reagent pair. Grossenbacher is alleged to teach that proteins produced in bacteria or that are otherwise in a denatured or non-native form can be renatured. The Examiner asserts Purchio as teaching that high level expression of eukaryotic proteins in CHO cells may lead to unnatural crosslinking of disulfide bonds, and that acidification can be used to quench a reduction reaction.

To summarize the rejection, the Examiner states that although Merli teach renaturation of protein produced by prokaryotic cells, Purchio teaches that recombinant proteins produced in eukaryotic cells may also be inactive due to unnatural crosslinking of disulfide bonds, so one of ordinary skill in the art would be motivated to optimize protein produced in eukaryotic cells as well. Since Grossenbacher evidences it was standard in the art to experimentally determine correct refolding of non-native proteins from different sources in order to reform

disulfide bonds by oxidizing agents and to purify such proteins, and Thomas teaches redox reagent pairs, one of ordinary skill would be motivated to optimize such conditions for a soluble TNF receptor Fc fusion protein produced by mammalian cells (as in Beutler), since such a Fc fusion protein is desirable as a therapeutic agent. Applicants traverse this rejection and submit that the cited references, taken together or in any combination, fail to establish a prima facie case of obviousness. As will be shown below, one of ordinary skill would simply not be motivated to combine the cited references to reach the claimed invention. In fact, the cited art as a whole teach away from the pending claims.

Nothing in any of the references teaches or suggests that a soluble form of a recombinant TNF receptor that has been produced in mammalian cells and/or that is glycosylated should be refolded by contacting it with a reduction/oxidation coupling reagent. In fact, nothing in any of the cited references suggests that one would need to do this. Merli, which teaches both *E. coli*-derived and CHO-derived soluble p55 TNF receptor, only subjects the *E. coli*-derived TNF receptor to refolding conditions. The CHO-derived TNF receptor of Merli is used only as a control- it is referred to as "std CHO" (p. 86, col. 1). This "std CHO" was then used as the control in an SDS PAGE analysis of large-scale renaturations (Figure 5), and in biological activity assays (Figure 6 and Table 2). The authors concluded "The data on the biological activity and, even more interestingly, those obtained with the structural probes suggest that all the purified sTNF-RI is correctly folded." (p. 90, col. 1) Because one of the gold standards for assessing refolding of the *E. coli*-derived TNF receptor was CHO-derived receptor, this would indicate that the CHO-derived receptor was considered correctly folded.

Purchio does not contradict this teaching of Merli. Purchio noticed that the CHO cell line that they created to express TGF-beta secreted primarily mature TGF-beta, but also secreted a small fraction of a larger rTGF-beta precursor (p. 12, lines 29-32). When they looked closed at this precursor contaminant, they found that it consisted of pro-TGF-beta, mature TGF-beta, and the pro region of the prescursor interlinked by a disulfide bond to the mature TGF-beta (p. 12, lines 39-43). The authors could not definitively explain this observation; they state that it could be unnatural disulfide bonds occurring as an expression artifact, or that it may be an important intermediate in TGF-beta processing (p. 12, lines 48-50). These results of Purchio simply does not teach or imply that all recombinant proteins expressed in CHO cells will have unnatural disulfide bonds. Purchio only teaches that that higher molecular weight rTGF-beta *precursor* had an unusual disulfide bond, and that it might be unnatural or that it might be an "important intermediate".

Furthermore, there would be no reason to combine Purchio, which deals with an artifact of TGF-beta expression in CHO, with Merli, which deals with refolding of *E. coli*derived TNF receptor. Nothing in either of these references, or in the cited art, suggests that there would be an unusual TNF receptor precursor as there was an unusual TGF-beta precursor in Purchio. Even if one did combine them (and Applicants do not concede that one of ordinary skill would), one still would not reach the claimed invention because there is still no suggestion in either Merli or Purchio, or indeed in any of the cited art, to refold a soluble form of a recombinant TNF receptor that has been produced in mammalian cells and/or that is glycosylated by contacting it with a reduction/oxidation coupling reagent.

The remaining references in combination with Merli and Purchio, also cannot render the claimed invention obvious. Grossenbacher relates to thrombin inhibitors expressed in bacterial hosts, and describes known methods of denaturing and renaturing. Thomas relates to TPO expressed in either mammalian (293 and CHO cells; examples 1 and 2) or *E. coli* cells (examples 3 and 4). Significantly, in Thomas, only the non-glycosylated TPO expressed in *E. coli* was refolded in a refolding buffer containing oxidized and reduced glutathione (col. 23-24). Beutler does relate to a TNFR:Fc fusion protein expressed in mammalian cells, but nothing in Beutler suggests contacting such a protein with a reduction/oxidation coupling reagent.

In fact, taken together, the cited art as a whole teaches away from the claimed invention. The references cited only serve to highlight the suggestion in the art that expression of recombinant proteins in mammalian cells (e.g., glycosylated) was a way to avoid the need to reduce and refold that was often required when such proteins were expressed in the standard bacterial system, *E. coli*. This teaching is particularly highlighted by the contrast in Thomas between the protocols for obtaining biologically active TPO from mammalian cells (no reduction protocol) with that for obtaining biologically active TPO from *E. coli* (a refolding protocol entailing reduction). The instant case is similar in these respects to the case of *In re Hedges*. There, in reversing a rejection for obviousness, the Federal Circuit said "On balance, Hedges proceeded contrary to the accepted wisdom. This is "strong evidence of unobviousness". "*In re Hedges*, 228 USPQ 685 (Fed. Cir. 1986), citing *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 220 USPQ 303, 212 (Fed. Cir. 1983), *cert. denied*, 105 S. Ct. 172 (1984), citing *United States v. Adams*, 383 U.S. 39. As in *Hedges*, the inventors here proceeded contrary to what was taught and suggested in the cited art to reach their claimed invention.

In view of the above, the cited references do not establish a *prima facie* case of obviousness, and the rejection should be withdrawn.

CONCLUSION

Applicants submit that the presented claims are in condition for allowance. A favorable action is earnestly requested. Applicants' attorney invites the Examiner to call her at the number below if any issue remains outstanding.

Respectfully submitted,

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